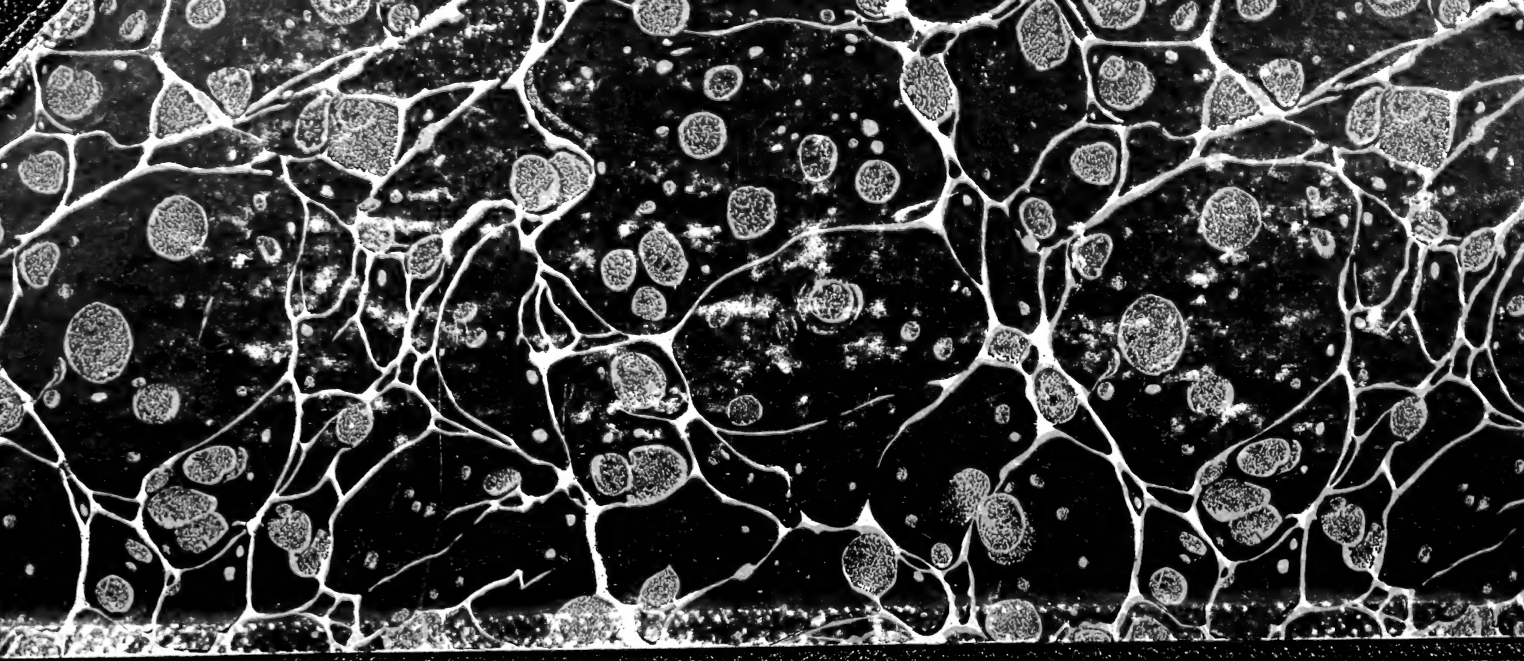

UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 1023

Contribution from the Bureau of Chemistry
W. G. CAMPBELL, Acting Chief

Washington, D. C. ▼ January 9, 1922

THE RELATIVE TOXICITY OF STRYCHNINE TO THE RAT.[1]

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PURPOSE OF INVESTIGATION.

Strychnine, which heretofore has been regarded with little favor as a poison for rats, was selected as the second of a series of such substances to be studied in the Bureau of Chemistry. The work was designed primarily to ascertain the toxicological conditions under which strychnine will prove efficient and to determine the minimum amounts of this comparatively expensive drug which are required to kill rats.

RESULTS OF PREVIOUS INVESTIGATIONS.

The literature pertaining to the pharmacology of strychnine is very extensive, but only a few investigators have chosen the rat as an experimental animal. Allard (1)[2] cites Savory, who is said to have

[1] The experiments discussed in this bulletin constitute the second of a series dealing with substances employed as rat poisons. The first, on barium carbonate, was issued in 1920 as Department of Agriculture Bulletin 915.

[2] The figures in parentheses refer to "Literature cited," at the end of this bulletin.

killed a rat with 1 milligram of strychnine injected per rectum. Hunt [3] found the lethal subcutaneous dose to be 3 milligrams per kilo, and reported (12) that the toxicity of strychnine remained constant under thyroid feeding, a condition which appreciably influenced the susceptibility to some other substances. No references giving the oral lethal dose of strychnine were found, although many articles deal with its use as a rat poison. Rucker (21) states that poisoned grain is of little use because of its bitter taste. Boelter (3) says that "with a poison such as strychnine as the active ingredient, mice, as a rule, die on the spot, while rats die in their holes."

In a previous communication (23) it was shown that the efficient concentration of barium carbonate in a rat bait is equal to three times the lethal dose contained in a meal of average size. This method of calculation, however, does not seem to be applicable to strychnine at present, because of the fact that, according to previous investigators, rats refuse to eat bait containing it. It has been shown also (23) that soft, mealy food is consumed by rats more quickly than grain or dog biscuit. Whether or not the rapid eating of a bait has any practical significance other than to favor the chances for the ultimate consumption of a fatal dose of a particular strychnine bait apparently has yet to be determined.

PHARMACOLOGICAL ACTION OF STRYCHNINE.

The story of the action of strychnine is one of the most interesting in the field of comparative pharmacology. Although strychnine possesses the properties of a general protoplasmic poison, it is important chiefly for its marked selective action upon the central nervous system. Its ability to pervert the function of the segmental reflex mechanism is general in the case of all vertebrates thus far studied. The earthworm (13), the simplest form in which neurone synapses have been described, has also been shown to give the typical strychnine spasm. This perversion, frequently termed the abolition of reciprocal innervation (27), consists in the destruction of that function which permits the release of opposing muscles when certain other muscles perform a motion. Under the influence of enough strychnine, all musculature tends to contract simultaneously, producing a muscle-bound condition. The position assumed by the poisoned animals is that which results from the action of the stronger or sometimes the more advantageously situated sets of muscles.

While this perversion of function has been preserved in vertebrates throughout evolution, from time to time certain changes have

[3] Personal communication from Dr. Reid Hunt, Boston, Mass.

occurred. The size of the dose of strychnine required to produce death tends to decrease as the evolutionary scale is ascended. Unexplainable differences, however, are reported between the more closely related species such as rats and mice (Table 1). On the other hand, a low resistance to strychnine is found (25) in animals having a higher morphological type of pyramidal tract in the spinal cords (rabbits, cats, and dogs).

TABLE 1.—*Species tolerance to strychnine salts (minimum lethal and sublethal subcutaneous dose).*

Animal.	Usual sublethal dose, per kilo.	Usual lethal dose, per kilo.	Citation.
	Milligrams.	*Milligrams.*	
Rat	2.5	3.0 to 3.5	Table 4.
Do.	2.0	3.0	Hunt (12).[1]
Mouse	.8	1.2	Do.
Do.	.76	.77	Falck (6).[2]
Do.	.....	1.5	Page 14.
Guinea pig	3.4	3.41	Falck (6).[2]
Do.	2.5	3.0 to 3.5	Hale (9).
Do.	.....	4.4	Hatcher and Eggleston (11).
Rabbit	0.4	.5	Hare (10).
Do.	0.45 to 0.50	0.55 to 0.60	Meltzer and Salant (16).
Do.	.....	.57	Falck (5).[2]
Cat	0.35	.40	Cutler and Alton (4).
Do.	.....	0.32 to 0.384	Hatcher and Eggleston (11).[3]
Dog	.....	0.35 to 0.42	Do.
Do.	0.30	.....	Hale (9).
Ground squirrel	0.6 to 0.7	0.7 upward.	Table 8.

[1] Personal communication from Dr. Reid Hunt.
[2] The salt administered by Falck was the nitrate. All other observers used sulphate.
[3] Hatcher and Eggleston (11) gave their dosages in terms of the alkaloid, but actually the sulphate was administered. The data given here have therefore been recalculated. The smaller dosages of these authors are for a solution containing 0.1 per cent of the alkaloid, the higher dose for 0.01 per cent solution.

The results from the type of experimentation shown in Table 1 are subject to fewer disturbing factors, so far as strychnine is concerned, than those from either the intravenous or gastro-intestinal methods of administration (11). These data, therefore, may be taken as representing the true species tolerance.

That the temperature of the environment also is an important factor is shown by the results of experiments on cold-blooded animals, in which it is easily possible to obtain a range of temperature amounting to 25° C. (29). Spasms occur more readily in frogs kept at 5° and at 30° C. than in those kept at 15° C. (7). The type of spasm also varies with the temperature. While frogs held at low temperatures have periods of long-continued tonic spasms, lasting sometimes for days, and separated by intervals of incomplete relaxation, those held at 30° C. undergo short spasms with more complete intervening relaxation. The short spasm, with practically complete relaxation, therefore, is the type observed in the homothermal, or so-called " warm-blooded " animals, which have a temperature-maintaining mechanism. Hibernation may directly affect the toxicity of and the behavior toward the drug, thus offering a possible explanation

of the discrepancies between the results of various observers (19), (30) on the hedgehog. Different batches of frogs sometimes vary in sensitivity toward strychnine. Neither the relation of this variation to the temperature and season nor the range between the convulsive and lethal doses has been determined.

The respective tolerance for any species of the adult homothermal mammals thus far studied has not varied markedly, the data reported in the literature by investigators who employed accurate methods agreeing very closely. Hunt and Seidell (12) have shown that while the tolerance of rats and mice to morphine varies with the dietary conditions, the toxicity of strychnine remains constant. Judging from the reliable data in the literature, no variation in the toxicity of strychnine caused by either diet or season occurs in other "warm-blooded" animals. In fixing the lethal dose, however, it would be advisable to perform many series of experiments, under suitable conditions, to rule out individual dietary and environmental factors.

Another modification which has occurred in the course of evolution is a tendency for a decrease in the number of spasms preceding death. Lower vertebrates, certain low mammals (24), and some very immature higher mammals (2), (5), (6), (15), (25) have multiple spasms preceding death. In certain immature mammals of the higher order a wide range between the convulsive and lethal doses exists. Very young animals are comparatively resistant to asphyxia and recover their ability to breathe more easily than mature members of the same species. On this account they can endure more successfully a large number of convulsions. This explanation, however, probably is not entirely adequate, for the reason that other characteristics differentiate the immature from the mature mammals here considered.

The size of the effective dose changes with the age of the mammals of certain species (25). At birth the mouse, rabbit, cat, and dog possess a natal immunity against the fatal action of strychnine, in this respect resembling the lower forms of animal life. Upon further development, however, this resistance is lost. After the decline of this resistance in the mouse a post-natal immunity, which reaches its limit about the time of sexual maturity, develops. The guinea pig also develops such a post-natal immunity, but, owing to its relatively mature state at birth, the decline of the natal immunity seen in other animals presumably is eclipsed by the period in utero. The cat, dog, and rabbit apparently develop no post-natal immunity, the adult lethal dose remaining at about the level to which the natal resistance declines.

Strychnine differs from many other poisons in that the body disposes of it very rapidly. The fact that small amounts of strychnine may appear in the urine for several days after its administration

does not necessarily signify a protracted period of danger or a marked increase in susceptibility of the individual to immediate subsequent poisoning. Hatcher and Eggleston (11), who have shown that the protective mechanism of the animal consists chiefly in the ability of the organism quickly to destroy this drug within the tissues, found the following coefficients of disposal, which are expressed in terms of the percentage per hour of a minimum subcutaneous lethal dose: For the cat, 12 to 25; for the dog, 14 to 17; and for the guinea pig, 18 to 57. These coefficients represent the conditions found when the strychnine is maintained somewhere in the neighborhood of the lethal concentration and not absolute hourly decrements when only a single subcutaneous lethal dose is given.

Under ordinary circumstances, in case of survival of the animal, strychnine is completely absorbed from the gastro-intestinal tract (12), but the exact rôle played by the stomach has not yet been determined. Although this organ is not endowed with general absorptive properties, it has been shown (22) that strychnine can be absorbed from the mucosa of Pawlow stomach fistulæ in dogs. However, a fatality from the administration of the minimum oral lethal dose of strychnine to an animal, such as a dog or cat, which usually has an empty stomach, in which the absorption of the entire dose is through the stomach mucosa, remains to be demonstrated. Nevertheless, it is possible that the absorption of the effective portion of the dose may occur in some instances chiefly or entirely through the gastric mucosa, particularly when the dose is very large.

TABLE 2.—*Relation of the oral to the subcutaneous lethal dose of strychnine sulphate.*

Animal.	Oral lethal dose per kilo.	Subcutaneous lethal dose per kilo.	Ratio.	Citation.
	Milligrams.	*Milligrams.*		
Guinea pig	[1] 44.0	[1] 4.4	[3] 10 to 1	Hatcher and Eggleston (11).
Rabbit			7 to 1	Nobecourt (18).
Cat	[2] 4.48	[2] 3.84	[2] 1.20 to 1	Hatcher and Eggleston (11).
Dog	[2] 5.4	[2] 4.6	1.20 to 1	Do.
Ground squirrel			[4] 5 to 1	S. E. Piper (17).

[1] Calculated from authors' data (Table 1).
[2] Calculated from authors' data, 0.0128 per cent strychnine sulphate solution used (Table 1).
[3] Approximate.
[4] Stomach to cheek-pouch ratio for poisoned grain.

In animals which can be obtained with empty stomachs, such as the cat and dog, the ratio of the oral lethal dose to the true species tolerance is very small, whereas in rabbits and guinea pigs, which have food continuously present in the stomach, it is extremely high. Presumably the rat would fall automatically into the second class, as the poison is to be administered in the food. This extreme point of

view must be taken to eliminate the influence of occasional adverse circumstances, such as food within the stomach ingested before the poisoned bait is eaten.

A large area of squamous mucosa occurs in the cardiac end of the stomach of the rat as a continuation from the œsophagus. If this has a favorable influence upon lowering the oral lethal dose it has not yet been shown. Such an influence should be considered, because much less strychnine is required to kill ground squirrels (*Citellus beecheyi*) when taken into the cheek pouches on grain[4] than when taken into the stomach either on grain or in solution.

Death from strychnine is due to asphyxia, which is the result of respiratory paralysis, the heart usually beating for some time after the spasms have abated. If a lethal dose has been given to an adult higher mammal, several spasms as a rule are sufficient. If the animal recovers from the initial spasm, it usually dies from a second or even a third. Instances of multiple and repeated attacks of spasms deserve investigation from the standpoint of their prevention by suitable environment. Life may be prolonged and sometimes saved by deep anæsthesia. Githens and Meltzer (8) obtained some recoveries from twice the lethal dose by the use of intravenous injections of Ringer solution, together with intratracheal insufflation. Presumably the advantage was double in that strychnine was eliminated at a relatively rapid rate in the urine on account of the diuresis and destroyed in the tissues at the usual rate. In the meanwhile the artificial respiration saved the life of the animal until the strychnine had been reduced below the absolute lethal amount.

Because of the relative suddenness of intoxication and the quick termination, remedial therapeusis in man ordinarily fails of an opportunity for use. Severe spasms in themselves are not necessarily a cause of death, as is shown by their occurrence in tetanus. Practically, however, it is possible to hasten death by producing them, or delay it, either by warding them off or by shortening them by the use of anæsthetics. For this reason therapists have recommended anæsthesia, together with absolute quiet. The reflexes resulting from the least noise or draft of air may inaugurate the vicious cycle of spasms.

On the other hand, the outcome in children, who are supposed to be more resistant than adults (28), seems less hopeless. A child (4) who apparently recovered after receiving an intraspinal injection of magnesium sulphate behaved in many ways like the infant animals. Even though lethal doses be administered to young animals, death is delayed. Life is prolonged thereby until suitable remedies

[4] Unpublished results of S. E. Piper, Bureau of Biological Survey, U. S. Department of Agriculture.

can be applied. Cutler and Alton (4) also successfully treated grown cats by the same method for exactly the minimum fatal dose of strychnine. The time element renders the practical application of this less favorable in adults than in infants. The use of magnesium sulphate is accompanied with some danger, which also precludes the use of this procedure unless previous experience in its application has been had.

EXPERIMENTAL PROCEDURE.

Throughout the course of this investigation strychnine has been considered from the angle of comparative pharmacology, the reason being that at some future time this drug may be more widely employed in poisoning animals than at present. Moreover, the relation of one species to another offers a more tangible basis for illustration than abstract data representing the lethal dose for rats. At the same time this is a very suitable plan from the aspect of the drug itself in that it has been used extensively as an experimental means in biology.

The brown and the white strains of *Rattus norvegicus*, as well as a few mice and ground squirrels (*Citellus richardsoni*), were employed for the experiments herein reported.

Two preparations of strychnine, one of free alkaloid and one of sulphate, tested and found to be free from brucin,[5] were used. The free alkaloid was passed through a sieve having 100 meshes to the inch. Two preparations of the adsorption combination of strychnine sulphate with diatomaceous or infusorial earth, found by the Kjeldahl analysis[6] to contain the amount of the alkaloidal salt stated on the label, were used. Standard 0.1 per cent strychnine sulphate solution was made up in physiological salt solution on the day of the experiments, lower dilutions being made as required from a freshly prepared 0.1 per cent solution. Thick cornstarch paste was taken to maintain a uniform suspension of the free alkaloid and of the infusorial-earth preparations. These suspensions were prepared in a small mortar, made up to volume in a 100 cc. volumetric flask by the starch paste and water washings from the mortar and thoroughly shaken. Amaranth, which in the concentration employed did not form a visible precipitate with strychnine, was used in some experiments for tracing the course of strychnine in the gastro-intestinal tract.

Injections into the stomach were made with a small hard-rubber urethral catheter, fitted over the outlet of a record syringe. The measurement of the starch-paste suspension therefore was by differ-

[5] H. E. Buc, formerly of the Bureau of Chemistry, made the tests.
[6] L. J. Jenkins, of the Bureau of Chemistry, made the analysis.

ence. Suitable amounts of starch-paste suspension containing the suspended alkaloid were driven into a Kjeldahl distillation flask through this tube by the syringe, and were found by analysis to contain practically the theoretical amount of nitrogen.[7]

Preliminary light ether anæsthesia was employed in all the stomach tube and in a few of the subcutaneous injection experiments. The anæsthesia passed off very soon. Subcutaneous injections were made under the skin of the abdomen. Young white rats of known age raised in the laboratory were used for testing the effect of age upon the size of the lethal dose of strychnine. All rats were put in small cages which were inverted to give the animals a better opportunity of grasping the wire netting with their toes. This procedure was adopted because it was found that rats kept in large cages sought the wire netting to endure their period of strychninization. Rats kept on metallic-sheet cage bottoms very easily lost their footing and had more frequent spasms. Autopsies were sometimes performed to ascertain the course of material injected, as well as the precision of some of the injections.

Table 3 gives data showing the relation of the alkaloid to its salts, as well as solubility data. Under some circumstances it might not be necessary to regard small differences in the percentage of strychnine in any one of its salts, but in this case it merits consideration.

TABLE 3.—*Relation of strychnine to its salts and their solubility in water.*

Preparation.	Molecular weight.	Contained strychnine.	Equivalent of strychnine sulphate.	Solubility in water.[1]
		Per cent.	*Per cent.*	
Strychnine sulphate	856	78	100	1 in 32.
Strychnine nitrate	397	84	108	1 in 42.
Strychnine alkaloid	334	100	128	1 in 6,420.

[1] U. S. Pharmacopœia, IX.

RESULTS OF EXPERIMENTAL WORK.

TOXICITY OF STRYCHNINE ADMINISTERED SUBCUTANEOUSLY.

The results of the subcutaneous administration of strychnine sulphate (Table 4) shows that the rat is exceedingly resistant to strychnine, being only slightly less immune than the guinea pig and very much more resistant than the rabbit, cat, dog, and ground squirrel (Table 1). The fact that the wild rat appears to be slightly more resistant than the white rat may be only apparent and due to the experimental error. The mortality in the white rat experiments, which make up Series F and which were performed on different

[7] L. J. Jenkins, of the Bureau of Chemistry, made the analyses.

days, varied from 20 to 50 per cent. The mortality from the experiments on wild rats (Series D and E) falls within this range. Moreover, the white rats received slightly larger doses, since they ordinarily possess much more body fat and have more material in the gastrointestinal canal than the wild rats in captivity. Moreover, the white rats used were raised under the best conditions, while the skin of wild rats frequently becomes thickened and tough, which may have had some part in delaying absorption of the drug.

TABLE 4.—*Toxicity of strychnine sulphate for rats administered subcutaneously in 0.1 per cent concentration.*

Series.	Rats.		Light anæsthesia.	Weight range.	Dose per kilo.	Survivals.	Fatalities.	Mortality.
	Number.	Kind.						
				Grams.	*Milligrams.*			*Per cent.*
A	8	Wild	Yes	140 to 290	2.0	8		
B	7	Wild	Yes	168 to 300	2.5	7		(1)
C	2	White	No	251 to 284	2.5	2		(1)
D	10	Wild	Yes	90 to 330	3.0	8	2	20
E	6	Wild	No	140 to 290	3.0	5	1	[2] 17
F	43	White	No	175 to 370	3.0	26	17	[3] 39
G	13	White	No	203 to 290	3.2	3	10	77
H	14	Wild	Yes	126 to 404	3.5	4	10	[4] 73
I	12	Wild	No	85 to 340	3.5	1	11	[5] 92
J	5	White	No	200 to 354	3.5	1	4	80
K	9	White	No	200 to 296	4.0		9	100

[1] No spasms occurred, but some animals were hyperexcitable.
[2] Only two rats had spasms.
[3] These animals were used for disposal tests, *vide infra*.
[4] Prespasm period, average 21 minutes; premortal period, 13 to 60 minutes, average 35 minutes.
[5] Prespasm period, average 17 minutes; premortal period, 17 to 55 minutes, average 33 minutes.

It is not evident that preliminary transient etherization played any rôle in the prevention of fatalities. While there is a slight difference in the total percentages between Series H and I, the other experimental data of those succumbing in both series show a remarkable agreement. Series D and E showed the reverse, the mortality being practically the same, but not the time relations.

The only way to reconcile these slight differences is by regarding the experimental error as sufficient to deceive the experimenter unless he guards against a too strict interpretation of numerical data. This factor of etherization was tested for the purpose of serving as a control to the stomach tube experiments, in which it was necessary to employ this preliminary step. These experiments show that etherization does not exert a lasting effect. If etherization is to be used therapeutically, therefore, it should be tried simultaneously with the appearance of spasms.

Under the conditions of the experiments the usually certain lethal dose is fairly constant and lies, within a narrow range, between 3 and 3.5 milligrams per kilo. The results obtained in experiments

elsewhere reported, in which the cages were not inverted, were much less concordant. As a further test, several rats were shaken from the sides of the cages to which they were tightly holding, with the production of fatal spasms. These observations on the rat seem to be in harmony with the fact that but few investigators have mentioned the conditions under which their animals were kept during the experimental period. Different conditions of experimentation may account for a variation in the size of the minimum lethal dose and the occurrence of convulsions from a sub-lethal dose. No tests were made, however, on other species for the purpose of reproducing some of the inconcordant data in the literature. Although rabbits, guinea pigs, and dogs are unable to grasp as well as climbing animals (cats and rats), it would seem advisable in all cases to select an environment offering the opportunity obtaining in nature.

Since it is possible to produce or hasten death by producing spasms, a few experiments were conducted to see if life could be prolonged by enforcing quiet. Several rats were given 4 milligrams of strychnine sulphate per kilo and placed on a slippery glass plate. In due time a sudden jump in the air and violent tonic convulsions resulting in death occurred. Previously the rats had stood practically motionless except for the movements of respiration. These results show the impracticability of securing survivals from more than a lethal dose by maintaining a volitional but forced quiet, because uncontrollable and involuntary motions will occur and start the vicious cycle of convulsions. Nevertheless, adult rats may be more or less influenced by the environment of and procedures resorted to in the experiment, and low results may be obtained.

In the experiments which were performed at various times in the year and on rats on different diets, no variation in the size of the lethal dose which could be assigned to dietary seasonal influences was obtained.

TOXICITY OF STRYCHNINE ADMINISTERED ORALLY.

Tables 5 and 6 show the results of administering strychnine sulphate and strychnine alkaloid orally.[8] The attempt to obtain rats with food in the stomach was not always successful, particularly in the case of wild rats. While some fatalities presumably were caused indirectly by the absence of food from the stomach, as well as by accidentally forcing the injected material beyond the pyloris, this error is automatically ruled out when the percentage of deaths is near the maximum. The data in the two tables agree in general, in

[8] For various reasons it was not feasible to depend upon feeding experiments. The reliability of the stomach tube as a means to obtain accurate data was shown in a previous report (23).

that from 20 to 25 milligrams of strychnine alkaloid or its equivalent in the alkaloidal salt is required to insure a large percentage of fatalities.

TABLE 5.—*Toxicity of strychnine sulphate administered in solution by stomach tube to lightly anæsthetized rats.*

Number of rats.	Kind.	Weight.	Strychnine sulphate solution.	Dose received, per kilo.	Survivals.	Fatalities.	Mortality.
		Grams.	*Per cent.*	*Milligrams.*			*Per cent.*
13	Wild	120 to 406	0.1 to 1.0	2.8 to 6.0	13	0	0
14	Wild	66 to 362	1.0 to 1.0	10.0	9	5	35
13	Wild	60 to 334	.5 to 1.0	15.0	4	9	69
13	Wild	46 to 370	.5 to 1.0	20.0	3	10	77
4	Wild	148 to 220	1.0	25.0	1	3	75

TABLE 6.—*Toxicity of strychnine (alkaloid) administered by stomach tube to lightly anæsthetized rats as 1 per cent suspension in starch paste.*

Number of rats.	Kind.	Weight.	Dose received.		Survivals.	Fatalities.	Mortality.
			Strychnine alkaloid, per kilo.	Calculated as strychnine sulphate, per kilo.			
		Grams.	*Milligrams.*	*Milligrams.*			*Per cent.*
26	Wild	110 to 404	7.5 to 12.0	9.6 to 15.4	11	15	58
4	White	132 to 205	10.0	12.8	3	1	25
5	White	172 to 208	15.0	19.2	1	4	80
18	Wild	84 to 385	15.0	19.2	3	15	83
15	Wild	62 to 360	20.0	25.6	1	14	93
9	Wild	160 to 473	25.0	32.0	0	9	100

TOXICITY OF DIATOMACEOUS EARTH PREPARATIONS OF STRYCHNINE.

The results of the administration by stomach tube of strychnine sulphate combined with diatomaceous earth (so-called tasteless preparation of strychnine) appear in Table 7. In this adsorbed form and concentration the toxicity of strychnine was reduced more than 50 per cent.[9] Since the alkaloid in this combination is not known to be liberated in an acid medium, it probably was not liberated in the stomach. Obviously diatomaceous earth would be an imperfect antidote for strychnine poisoning on account of the uncertainty of its opportunity to become attached to previously ingested strychnine, as well as the uncertain stability of the substance when formed.

[9] These results were obtained with preparations containing from 6 to 8 per cent of strychnine sulphate. It is possible, however, that much larger quantities of strychnine could be placed in preparations of this type, with no practical effect upon their palatability, but with an increase in the potency of the strychnine actually present. This possibility may be explained by the supposition that the ease of liberation of the strychnine in the beginning is greater when large amounts of strychnine are present.

The only conceivable advantages in the use of a preparation of this sort are its highly probable nonabsorption in the stomach, and the absence from it of taste. Presumably the rat would receive no immediate physiological warning, since no strychnine would be liberated to be absorbed by the stomach. The amounts which would be surely lethal, however, are large and the cost of the commercial article is so great that the practicability of its use for rats is open to question at present.

TABLE 7.—*Toxicity of strychnine sulphate diatomaceous earth preparation to wild rats.*

Number of rats.	Weight.	Dose of strychnine sulphate per kilo.[1]	Survivals.	Fatalities.	Mortality.
	Grams.	*Milligrams.*			*Percent.*
8	126 to 328	10	[2] 8		
2	186 to 192	20	[3] 2		
5	105 to 360	25	[4] 4	[4] 1	20
2	130 to 136	35		[5] 2	100
2	234 to 300	40	1	[5] 1	50
9	93 to 300	50	4	[6] 5	55

[1] Material was suspended in starch paste so as to contain 1.0 per cent of the alkaloidal salt.
[2] Only 2 were spastic.
[3] Both were nervous.
[4] All were spastic.
[5] Fatalities occurred after second hour.
[6] Five died within 24 hours.

EFFECT OF AGE OR DEVELOPMENT UPON TOXICITY OF STRYCHNINE.

In experiments reported elsewhere (25) it has been shown that as the rat develops it passes through stages indicating in a functional way the development of its nervous system. Early during the nursing or crawling stage the tolerance to strychnine is high. There is also a latitude between the minimum convulsive and the minimum lethal subcutaneous dose. By the time the animal acquires some physical dexterity the lethal dose falls to about the level of the convulsive dose. These rats then have only a few spasms preceding death, while in the crawling stage when a lethal dose is administered many spasms usually occur. About the time the rat is capable of beginning an independent existence (25 days) the resistance begins to rise, and ordinarily no convulsions occur until the lethal dose is reached. The highest subcutaneous dose survived by the rats in the crawling stage is 2 milligrams per kilo. Just after this stage is passed (in the weaning stage) the lethal dose is approximately 0.5 milligram per kilo. At about the time of sexual maturity it is 3 milligrams per kilo, or six times that of the weaning stage tolerance.

DISPOSAL OF STRYCHNINE.

In a series of experiments reported in detail elsewhere (26), it was found that the rat can always dispose of 33 per cent of a minimum lethal subcutaneous dose of strychnine in two hours; that about half

of the rats can dispose of 50 per cent; and that only rarely does one successfully dispose of 67 per cent. This was determined by injecting a series of rats with the minimum lethal subcutaneous dose, and at the end of two hours injecting those surviving with one of these fractional doses.

Coefficients of this magnitude are representative of the experimental conditions obtained when the drug is maintained somewhere near the lethal concentration, because strychnine may be found in the urine of various animals for several days after similar injections are made. Coefficients obtained in this manner, therefore, apparently can be applied only on a relative basis. To illustrate: The average coefficient of disposal is 50 per cent the first two hours. The amount disposed of the second two hours is presumably only 50 per cent of the 50 per cent which remained at the end of the second hour. At the end of the fourth hour, if the application of the coefficient were by absolute amounts, no strychnine would be left, whereas on a strictly relative basis 25 per cent would still be present. For this reason halving the coefficients obtained for 2-hour periods probably gives results slightly low for the first hour. The data, nevertheless, illustrate the marked ability of the rat to withstand this drug, providing the maximum sublethal concentration is not exceeded. An average rat, however, can eliminate a minimum lethal subcutaneous dose with certainty in four hours (26) and presumably in three hours, if the amount of strychnine is continuously kept near the lethal concentration.

Because of this rapid disposal it is possible for the rat to survive many times the lethal subcutaneous dose when it is administered by mouth, providing the amount present within the system at any one time does not exceed the maximum sublethal dose. This also explains why some animals (28) are extraordinarily resistant to strychnine by mouth. In comparing the oral toxicity of strychnine in one species of animals with that in another, ratios of potency of from 1 to 50, or even from 1 to 100, may be obtained. Analysis will show that the reason for so high a ratio may be that one of the animals used for the comparison is very sensitive and has an empty stomach, thus requiring only a small oral dose, while the other has a full stomach, retarding the absorption of the strychnine and increasing from 5 to 10 times the originally high species tolerance. Ratios of potency of this magnitude, therefore, must be considered as practical ratios only, rather than as the true indices of species resistance.

ABSORPTION FROM STOMACH.

In considering previously the absorption of strychnine from the stomach, the question of distinguishing between absorption by the stomach of a lethal dose of strychnine in the absence of food and in

the presence of food was raised. By using amaranth, which did not form a precipitate with strychnine in the concentration in which it was used, it was possible to trace the course of strychnine down the gastro-intestinal canal. In some instances the dye had penetrated into the duodenum or very close to the pyloris; in others the antrum of the stomach was filled by well-packed, semidry contents, the dye being in the cardiac and midportions of the stomach. Death in the second case must have been due to absorption directly through the stomach wall. The possibility that the food filtered out the coloring matter as the strychnine solution progressed seems untenable because of the remoteness from the pyloris and the fluid character of the dyed material in contrast with the semidry material in the antrum. Practically it would appear to make little difference whether death ensued by sufficiently accelerating or forcing a gastric absorption by a high concentration or by permitting a small amount to enter the small intestine, or by a combination of both. These results do not indicate a marked absorption of strychnine in the stomach, although death may ensue when the dose administered is very much greater than the subcutaneous lethal dose.

RELATIVE TOXICITY OF STRYCHNINE TO GROUND SQUIRRELS, MICE, AND RATS.

Table 8 shows the results of experiments on ground squirrels (*Citellus richardsoni*), the minimum subcutaneous lethal dose being 0.7 milligram per kilo. The data show also that the ground squirrel is four or five times more susceptible to the poison administered subcutaneously than the rat. This is one of the two pharmacological reasons which make the successful poisoning of the rat more difficult. The case of ground squirrels differs somewhat from that of rats, since it has been shown by Piper (17) that the poison is absorbed from the cheek pouches and that the lethal dose is also much less by this route than by the stomach. The range between the highest dose of strychnine survived and the lowest lethal dose in the experiments reported by Pierce and Clegg (20) for California ground squirrels (*Citellus beecheyi*) is so great that no accurate comparison of their data with those here reported can be made.

According to Falck (6), the toxicity of strychnine administered subcutaneously to the mouse is about four times greater than that here reported for rats. According to Hunt's data (Table 1) it is about two and five-tenths times more toxic. In the experiments herein reported large white and wild mice were tested with the same technique as that used for rats. It was found that 2 milligrams of strychnine sulphate per kilo injected subcutaneously proved fatal, 1.5 milligrams being somewhat less certain. For these particular mice strychnine was about half as toxic as it was for rats.

The lethal dose per os was approximately three to five times the subcutaneous dose. Both the subcutaneous and oral experiments bore out the inference to be drawn from Boelter's statement (3) that when administered orally it usually acts more quickly in mice than in rats. Owing to the great number of experiments required and the inability to obtain very large specimens, no attempt was made to study the effect of size, strain, and experimental technique upon the toxicity of strychnine to mice. Undoubtedly, however, the mice which have thus far been tried are more susceptible than rats and apparently they should be three or four times as easy to poison as rats.

TABLE 8.—*Toxicity of strychnine sulphate administered subcutaneously to male Richardson ground squirrels (Citellus richardsoni).*[1]

Date.	Weight of squirrel.	Strychnine sulphate solution injected.			Result.
1920.	*Grams.*	*Per cent.*	*Cc.*	*Milligram per kilo.*	
Aug. 6	160	0.05	0.32	1.0	Fatal spasm began in 28 minutes; dead in 30 minutes.
Sept. 8	280	.02	1.25	.9	Fatal spasm began in 11 minutes; dead in 12 minutes.
Do	339	.02	1.18	.7	Several mild spasms; partial ability to recover; dead in 18 minutes.
Aug. 6	184	.01	1.29	.7	Hyperexcitability only; lived.
Do	240	.01	1.44	.6	Lived.
Do	190	.01	.95	.5	Do.

[1] Acknowledgment is due Vernon Bailey, of the Bureau of Biological Survey, for procuring these animals.

STRYCHNINE BAITS.

White rats were given 1 per cent strychnine bait in the form of soft, mealy food containing about 20 per cent of fat. These rats were accustomed to such a diet, without the strychnine. That the bait was distasteful to them was evident from the way they rubbed their mouths on the floor and sides of the cage. Within about 15 minutes most of the rats were unusually excitable and some had sought the wire netting on the sides of the cages for support, indicating that part of the strychnine bait had been consumed and strychnine absorbed.

Obviously this muscle tenseness and excitability might cause the animal to become suspicious and cease eating. Strychnine baits of a type favoring rapid consumption are preferable. Although the food was left in the cages for more than 18 hours, only a few rats died. These results agree with those of practical tests showing that strychnine bait is not well eaten by rats. So much of the food was scattered that no attempt was made to estimate that which remained uneaten.

In a previous communication (23) it was shown that the average size of the meal consumed by hungry white rats was one one-hundredth of their body weight. Good practical poisoning results were obtained with barium carbonate by putting three times the amount necessary to kill (20 per cent) in the bait. If one-third of the average meal were consumed a fatal dose would be ingested. On this basis the efficient percentage of strychnine in a rat bait should be 0.75 per cent, or 1 part in 133 parts of bait. This concentration will be useful in the preparation of baits that are tasteless and rapidly consumed. Practically, however, it may be found that more strychnine will be required if such baits are not consumed quickly, or if the potency is decreased.

Table 9 gives a list of baits, worked out by practical tests, which seem to contain a very liberal amount of strychnine for all animals other than the rat. The rat, however, is five or six times more resistant to strychnine administered subcutaneously (Table 1) than the ordinary domesticated rabbit. If these data for the domesticated rabbit are representative also for the jack rabbit, the liberality with which this animal has received strychnine in its bait and the shortcomings of the rat bait will at once become apparent. The rat baits contain on the average but little more strychnine than the jack rabbit baits. The ground squirrel (*Citellus richardsoni*) is four or five times more sensitive to strychnine than the rat (Table 8). These animals are killed by relatively much less strychnine because strychnine is far more potent by way of the cheek pouches than when taken into the stomach. The difference between the concentration of strychnine in the ground squirrel baits and that in the rat baits, however, is not as marked as might be expected.

TABLE 9.—*Types of strychnine bait used for poisoning animals.*

Bait.	Strychnine.		Animal.	Equivalent or approximate amount of strychnine sulphate in dry bait.		Citation.
	Form in which added.	Ultimate form.		*Parts.*	*Per cent.*	
Barley	Sulphate	Sulphate	Ground squirrel.	1–480	0.21	Merriam (17).
Milo maize	Alkaloid	Alkaloid	Prairie dog	1–290	.35	Bi. 158.[4]
Do	Sulphate	do	do	1–370	.27	Bi. 158.
Do	do	do	do	1–360	.28	Bi. 163.
Alfalfa	do	Sulphate	Jack rabbit	1–257	.39	Bi. 163.
Milo maize	Sulphate or alkaloid.	Alkaloid	do	1–366 to 1–262	0.3 to 0.38	Bi. 163.
Barley	Sulphate	do	Ground squirrel.	1–290	.345	Pierce and Clegg (20).
Rolled oats	do	Sulphate	Rat	1–170 to 1–210	[1]0.59 to 0.48	Lantz (14).
Wheat	do	do	do	1–200	[2].50	Do.
Do	do	do	do	[1]1–100	[3].25	Rucker (21).

[1] Somewhat less on moisture basis.
[2] Estimate liberal.
[3] Allowing for presumable typographical error in original article; estimate liberal.
[4] "Bi." refers to mimeographed circular letter giving directions for making baits, issued by the Bureau of Biological Survey, U. S. Department of Agriculture.

SUMMARY.

The limit of tolerance of *Mus norvegicus*[10] to strychnine administered subcutaneously is from 3 to 3.5 milligrams of strychnine sulphate per kilo. Of the comparatively few species of mammals for which accurate data are available, the guinea pig alone possesses a resistance greater than this. The existing data indicate that while the lower orders of mammals, such as the Chiroptera (bats) (24) and Insectivora (hedgehog) (19) (39), are at times more tolerant than the guinea pig and the rat, at other times they are less tolerant.

The size of the subcutaneous lethal dose (species tolerance) remains constant when a certain definite experimental procedure is followed. General or mixed diets and seasons had no effect upon the species tolerance in the series of experiments reported in this bulletin.

The rat shows a marked ability to dispose of strychnine. Under favorable conditions the calculated disposal amounts to 1 milligram per hour (one-third of a minimum lethal subcutaneous dose). Practically, with the unfavorable experimental procedure used (discontinuous injections) it was slightly less than this.

Immature rats differ from adult rats, both in the toxicity of and reaction to strychnine, which seems to be correlated with the functional development of their nervous system.

The practically certain oral lethal dose of strychnine is from 20 to 25 milligrams per kilo of the free alkaloid, equivalent when calculated as the sulphate to from 25.6 to 32 milligrams. The ratio of the subcutaneous lethal doses to the oral lethal doses is about 1 to 8 or 1 to 9. The reason for this high ratio in rats would seem to be that when less than a lethal dose has been given the stomach and its contents directly or indirectly hinder absorption. On this account the animal can prevent the accumulation of a lethal amount within the system by the rapid disposal. When a lethal dose is administered by mouth the fatality usually occurs within several hours. The rat, therefore, must be overwhelmed by the drug; otherwise, it probably will survive. At times this certainly involves the play of a usually subordinate function, gastric absorption.

On the basis of its alkaloid content, the toxicity of two so-called tasteless preparations of strychnine (adsorption compound of diatomaceous or infusorial earth with strychnine sulphate) was found to be reduced by more than 50 per cent.

Mice are more susceptible to strychnine than rats, both to subcutaneous injections and to doses orally administered.

[10] It is to be hoped that the first opportunity presenting itself for a comparative test with *Mus rattus* will be accepted by those into whose hands these data fall. The supposition that the toxicity of strychnine will be the same in both cases is not warranted, although it is legitimate to infer that this is true in the case of barium carbonate.

Ground squirrels (*Citellus richardsoni*) are about four or five times more sensitive to strychnine administered subcutaneously than rats. This helps to account for the comparative difficulty in poisoning rats.

The percentage of strychnine which would make a successful rat bait can not be stated at present. After the factors of rapid consumption and palatability are solved, it should be possible to obtain good results with 0.75 per cent of the alkaloid in potent form in the formula. This percentage represents three times the fairly certain fatal dose in a meal of the average size. It is probable that any success obtained with lower concentrations represents instances of fatalities from amounts smaller than can always be considered certain or from larger amounts of bait than can always be relied upon to be eaten. Experiments herein represented should be continued, employing feeding tests with perfectly palatable strychnine baits, in order to determine how rapidly strychnine is absorbed from the particular bait. Since the absorption may be very rapid, an increase in the percentage of strychnine here given may be found expedient, even though the bait is eaten fairly rapidly.

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